VOL. XII, No. 3 MARCH, 1951

APR 23 1951

610.5
B73

BORDEN'S REVIEW

of

NUTRITION RESEARCH

DIETARY INTERRELATIONSHIPS:

II. The Metabolism of Methyl Compounds

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

DIETARY INTERRELATIONSHIPS:

II. The Metabolism of Methyl Compounds

By RUTH WOODS

By the end of 1949, just about ten years after the original proposal of the transmethylation concept*, at least two significant new aspects of this phenomenon had emerged†. These were (1) confirmation of an early indication that some degree of methyl synthesis actually occurs in the body and (2) that the need for dietary sources of methyl groups is markedly influenced by the folic acid and vitamin B_{12} contents of the diet, or by the amounts of these vitamins, particularly B_{12}, made available through storage in the liver from previous diets.

These new aspects of the methylation story provoked many additional questions: Where does the synthesis of methyl groups take place? Is it a function of bacterial metabolism in the intestine or can the body tissues themselves actually fabricate methyl-containing compounds? What are the building blocks from which methyl groups may be created? To what extent does methyl synthesis in the body contribute to the overall need for methyl groups? Are dietary sources of methyl groups still essential? Are all methyl donors equivalent or does methyl exchange involve reactions between specific compounds?

During 1950, preliminary answers to most of these questions began to appear with great rapidity from many laboratories. These have made it possible to interpret the often paradoxical results of the experiments described in the first part of this Review and have helped to round out the picture of biological methylations as it appears today.

Localizing the Biosynthesis of Methyl Groups:

The growth of animals on methyl-free diets, under certain conditions, and the demonstration of the formation of deuteriomethyl groups by animals whose body fluids contained deuterium-labeled water (D_2O) conclusively established the fact that labile methyl groups can be synthesized somewhere in the animal body. The probable key role of vitamin B_{12} and folic acid in this synthesis was also made evident through many of these studies. It now remained to establish whether body tissues are capable of this synthesis or

---

*cf. *Borden's Review of Nutrition Research,* October, 1942, "Labile Methyl Groups of Protein and Choline."

†c.f. *Borden's Review of Nutrition Research,* February, 1951, "Dietary Interrelationships I. "Anti-Anemia Factors and the Biological Methylating Compounds."

whether these methyl groups were merely being furnished through intestinal absorption of these materials resulting from activities of intestinal bacteria. This distinction was impossible to make on the basis of experiments already reported since, in all cases, both interpretations were conceivable.

Studies with Germ-Free Animals: At this point, fortunately, it became possible to settle the question beyond doubt through the use of one of the newest and most promising biological research tools developed — the germ-free animal.

The germ-free animal, described by Reyniers (1, 2) of Notre Dame, is a remarkable achievement. Animals of this type are obtained under completely sterile conditions by Caesarian section from pregnant mothers. They are reared in a sterile atmosphere, under germ-free conditions, and fed sterile food. Consequently, the animals themselves are completely germ free, even to their intestinal tracts which contain no bacteria or microorganisms of any kind, either good or bad. Through studies with such animals it becomes possible to evaluate the role of dietary components without the complicating factor of supplementary supplies of nutrients which may result from the activity of intestinal organisms.

Thus, the germ-free animal provided the ideal means for the crucial experiment designed to determine the place where methyl-synthesis occurs. The workers at LOBUND and the group at Cornell collaborated in a joint experiment to investigate this problem. The plan of the experiment was to maintain rats of the LOBUND* (germ-free) strain with D_2O in their drinking water under both germ-free and non-sterile conditions at the LOBUND laboratories. For comparative purposes, ordinary rats of the Rockland Farm strain were maintained at the Cornell laboratory under the usual laboratory conditions on the same diet as that used at LOBUND.

A preliminary report of the experiment was set forth in a general paper in *Science* by the Cornell group (3):

Two male, germ-free animals reared by the technique of Reyniers were maintained under germ-free conditions at LOBUND. Food and water were administered under sterile conditions and urine and feces were similarly collected. Sterilized drinking water containing 10 atom percent D_2O was given for 4 days, followed by 4 atom percent D_2O for the rest of the test period. The diet was essentially that already developed at LOBUND for rearing animals under germ-free conditions (containing all known vitamins except choline and including biotin, folic acid and B_{12}). At the end of various test periods, the germ-free animals were sacrificed under sterile condi-

*Laboratories of Bacteriology, University Notre Dame.

tions. The specially prepared sterile tissues, sterile urine and feces were then sent to the Cornell laboratories for analysis. At the same time, similar preparations from two other animals maintained at LOBUND under non-sterile conditions were also sent to Cornell. In order to compare the colony of animals under non-sterile conditions at LOBUND with the colony and experimental conditions at Cornell, two animals were maintained at Cornell on the same diet* as that used at LOBUND. In addition, two others were maintained at Cornell on the LOBUND diet plus extra supplies of B_{12}.

Determination of the synthesis of methyl groups was made by isolating choline from the tissues and excreta of the D_2O-fed rats and analyzing for the presence of deuterium. The results are summarized in Table 1.

From the data in this table it is apparent that methyl groups were indeed synthesized by the germ-free animals. The average deuterium level in the body water during the experiment had been calculated as approximately 2.5 atom percent. The enrichment of deuterium in the methyl groups of the choline isolated from the LOBUND germ-free rat after 23 days was 6.4 percent of that in the body water. In the 10-day experiment involving the rat under germ-free conditions, a value of 3.3 percent of that in the body water was obtained, an amount which is believed to agree quite well with that of the longer experiment. A somewhat higher value was obtained in the non-sterile LOBUND animals. Whether this difference was significant was questionable; it is possible that it may have reflected additional synthesis by bacterial microorganisms. The Cornell animals (non-sterile) on the LOBUND diet synthesized methyl groups to about the same extent as did the germ-free animals at Notre Dame. The extent of methyl synthesis by the Cornell animals was not increased by providing extra vitamin B_{12} above the amount contained in the LOBUND diet. These studies established conclusively that synthesis of methyl groups does take place and that it occurs in the tissues.**

In Vitro Studies: Coincident with the work of the Cornell group, independent investigations along entirely different lines in another laboratory provided further evidence for the synthesis of methyl groups by the tissues. Thus, at Western Reserve University, Sakami (13) engaged in an investigation of acetone metabolism, noted that this product gives rise not only to acetate, but to formate as well. Sakami reported further that when C^{14}-methyl-labeled acetone was administered to rats, appreciable radioactivity was found in the β-carbon of the serine isolated and also in the methyl car-

*Prepared at LOBUND and shipped air express to Cornell to eliminate any possible difference in source of dietary material.

**These experiments were not designed to shed light on the question of bacterial synthesis which possibly is still not precluded.

TABLE 1

DEUTERIUM CONTENT OF METHYL GROUPS OF CHOLINE

Rat No.		Experimental period	Change in body weight	Choline chloroplatinate		Trimethylamine chloroplatinate (A)		Deuterium in methyl groups of choline (B)*	Deuterium in body water (average) (C)	$\frac{B}{C} \times 100$
		days	*gm*	*mg*	*% Pt*†	*% Pt*†	*atom % excess D*‡	*atom % excess D*	*atom % excess D*§	
CUMC‖,¶	710	21	119-237	469	32.1	37.1	0.23	0.26	3.29	7.8
CUMC¶	707	21	109-186	429	31.7	36.7	0.19	0.21	3.08	6.9
CUMC	708	21	103-186	419	32.2	36.7	0.19	0.21	2.52	8.4
CUMC	709	21	105-225	462	31.2	36.8	0.21	0.23	3.01	7.8
LOBUND	1	21	183-253	533	31.4	36.9	0.19	0.21	2.20	9.6
LOBUND	2	21	180-282	579	31.2	37.0	0.18	0.20	2.27	8.8
LOBUND (germ-free)	5	23	294-322	661	31.4	36.8	0.14	0.16	2.44	6.4
LOBUND (germ-free)	6	10	308-289	632	31.4	37.2	0.09	0.10	2.99	3.3

* Calculated value: $B = \frac{10}{9} A$.

† Theoretical Pt content for choline chloroplatinate = 31.7%; for trimethylamine chloroplatinate = 37.0%.
‡ Determined with mass spectrometer (% error = ± 0.02).
§ Determined by falling-drop procedure (% error = ± 0.06).
‖ CUMC = Cornell University Medical College, Rockland Farms strain.
¶ Received additional 1.5 μg of vitamin B_{12} daily.

bon
sou
sho
act
in
the
tha
the
me
gro
me
cys
act
als
sin
de
in
gro
of
of
(4
gr
m
ch
th
ra
Fr
Th
rif
act
act
Th
ma
ca
ca
ca

bons of choline and methionine. This indicated that acetone may serve as a source of methyl groups. Using the β-carbon of serine as an indicator, it was shown that the β-carbon was not formed from formate via acetate, little activity being exhibited when C^{14}-methyl-labeled acetate replaced the acetone in the foregoing experiment. Comparison of the incorporation of C^{14} into the β-carbon of serine from methyl-labeled acetone and formate indicated that formate formation is a major mechanism of acetone metabolism under these conditions. The finding of C^{14} in the methyl groups of choline and methionine suggested the possibility that actual synthesis of labile methyl groups occurred via formate.

Further studies by Welch and Sakami (10) confirmed this possibility. They injected rats repeatedly with C^{14}-formate. The animals were then sacrificed and the protein content of the tissues analyzed. Appreciable radioactivity was noted in the methionine derivative isolated. In another experiment, rat liver slices were incubated in the presence of C^{14}-formate, homocysteine, dimethylaminoethanol, folic acid and vitamin B_{12}. Again, a radioactive methionine derivative was obtained. Radioactive choline chloride was also isolated from these rat liver slices.

Thus, evidence from two widely divergent experimental approaches — *in vivo* studies with germ-free animals and *in vitro* studies on rat tissues — simultaneously and independently achieved the same goal, that of clearly demonstrating the ability of animal tissues to synthesize methyl groups.

The Metabolism of Methyl Groups:

Early in the studies on transmethylation it was believed that labile methyl groups were transferred as intact units from one compound to another. Much of the evidence for this assumption stemmed from the isotope experiments of the Cornell group. In one of these studies, for example, duVigneaud *et al* (4) fed rats for three months a diet in which the only source of methyl groups was a specially prepared methionine labeled with deuterium in the methyl group. At the end of this period the animals were killed and the choline and creatine content of the tissues were examined. It was found that the compounds contained, in their methyl groups, 89% of the deuterium ratio present in the methyl groups of the administered isotopic methionine. From this, three conclusions were apparent:

1. Considerable transmethylation had occurred (from methionine to choline and creatine).
2. The methyl group was transferred intact rather than through degradation to an intermediate product (as, for example, formaldehyde) having only two hydrogen atoms instead of three (i.e. CH_2O vs. $-CH_3$). If such an intermediate had

been involved, the percentage of deuterium appearing in the choline and creatine could not have exceeded a maximum of 66% (i.e. ⅔). The fact that it was 89% indicated that the methyl group itself was directly involved. (The potential maximum of 100% might have eventually been reached if more time had been allowed for complete replacement of the body's original store of non-labelled choline and creatine).

3. Under conditions of this particular experiment*, very little synthesis *de novo* of methyl groups occurred, the majority of methyl groups in choline and creatine arising via transmethylation. This was indicated again by the high percentage of deuterium in the choline and creatine. This showed that the methyl groups of these compounds came from the labeled methionine in the diet. If the methyl groups had been synthesized, they would have no deuterium and consequently the percentage of recovered deuterium would have been far less than 89%.

Degradation of Methyl Groups: The investigation of methyl metabolism received renewed impetus with the synthesis of methionine containing radioactive carbon (C^{14}) in its methyl group. The synthesis of radiocarbon methyl, announced by Melville *et al* at Cornell (5) in 1947, made it possible to demonstrate for the first time the oxidation of the labile methyl group of methionine to carbon dioxide (6)†. In subsequent quantitative studies, Mackenzie and others of the Cornell group (7) found that as much as one-quarter of the radioactivity of C^{14}-labeled dietary methionine was recovered as expired CO_2 during the first day, the loss continuing afterward at a slower rate. This, together with the appearance of marked methyl groups in excreted creatine, creatinine and N'methylnicotinamide indicates a heavy, constant drain on the methyl supply.

Radiocarbon-labeled methyl compounds further made it possible to determine the intermediate products in the oxidation of $-CH_3$ to CO_2 and to confirm earlier speculations as to the probable nature and metabolic function of these intermediates. Thus, Mackenzie (8) studying the metabolism of C^{14}-labeled sarcosine (a methyl-containing amino acid) by liver slices and homogenates, was able to isolate radioformaldehyde and radiocarbon dioxide from these systems and to obtain evidence of the presence of radioformic acid (formate). He concluded, further, that most or all of the formate and CO_2 formed in the oxidation of the methyl group arise by way of formaldehyde. These studies provided the first direct evidence that formaldehyde

*As duVigneaud points out, the diet then used was very low in folic acid and vitamin B_{12}. In the light of present knowledge (*cf. Borden's Review of Nutrition Research,* February, 1951 "I. Anti-Anemia Factors and the Biological Methylating Agents") this may account for the minimum synthesis of methyl groups in this experiment. It may conceivably also explain the transfer mechanism involving minimum or no intermediates.

†Determined by collecting and precipitating exhaled CO_2 and analyzing for radio carbon; also by determining radioactivity of CO_2 from oxidized tissues.

is a normally occurring compound in the animal body, confirming the earlier suggestions and presumptive evidence of numerous other investigators. Mackenzie further stated that since collateral experiments had shown that the methyl groups of methionine and betaine contribute to sarcosine formation in the rat, "it follows that 'biologically labile' methyl groups as a class are sources of formaldehyde and formate in the body."

Simultaneously with the work of Mackenzie, Siekevitz and Greenberg (9) reported the formation of formate from the methyl groups of choline and methionine, as well as from the β-carbon of serine and the α-carbon of glycine.* These investigators also were able to show that formate is reduced to form the methyl group of methionine, both *in vivo* and *in vitro*. This confirmed a similar report by Welch and Sakami (10).

From these and other studies it is clear that methyl groups may undergo oxidative degradation to formaldehyde, formate and CO_2 in the body and, moreover, that these reactions are reversible in part so that formate or formaldehyde, but not CO_2, may be reduced to form new methyl groups. The implications, thereby, of methyl biosynthesis are obvious. That methyl groups also may be transferred intact, without degradation and resynthesis, however, has already been shown (4). As late as September, 1950, Siekevitz and Greenberg (9) stated "the partial oxidation of labile methyl groups may have no great significance for transmethylation in the animal organism." The fact that this reaction is reversible, however, as well as other pertinent evidence — all of which became available almost simultaneously during 1950 — served to strengthen the belief that, under certain conditions, biosynthesis of methyl groups does occur.

Precursors in the Biosynthesis of Methyl Groups:

Evidence for the biological synthesis of methyl groups by the animal body soon stimulated a new direction of investigation, namely a quest for the possible precursors of methyl groups in intermediate metabolism.

(Chemical Studies): duVigneaud and Verly (11) found that methyl alcohol may serve as a precursor of labile methyl groups. They noted also the formation of methyl groups in choline from formaldehyde and formate (12). This was in agreement with the work of Sakami (13) in his study on acetone metabolism which was later confirmed by Welch and Sakami (10) and by Siekevitz and Greenberg (9). Welch and Sakami having noted the formation of methyl groups from acetone (which compound was also shown to contribute to the formate pool) deduced, as a result of isotope studies,

*Siekevitz and Greenberg also postulated that the methyl groups of choline and methionine and the α-carbon of glycine are specific formate donors for the β-carbon of serine.

that the synthesis of methyl groups from acetone probably occurred via formate. This was confirmed by administration of radioformate to rats, as well as by incubation of rat liver slices in the presence of radioformate.* In each case, radiomethyl compounds (methionine or choline, or both) were subsequently isolated. Welch and Sakami stated, further, that studies were in progress on homogenates and on "the possible role of folic acid and vitamin B_{12} in the synthesis by various tissues of compounds containing methyl groups."

(Role of Vitamin B_{12}): Earlier studies reported in the previous issue of this Review had developed the concept of a relation between the anti-anemia factors and the need for dietary methyl supplies. Growth of animals on diets free of methyl groups had been reported possible in the presence of liver extracts containing the anti-pernicious anemia factor provided the diet included, along with other essentials, an adequate supply of folic acid. (The occurrence of growth is evidence of protein synthesis which, in turn, means synthesis of the essential amino acid constituents, including methionine.) These studies were repeated by Stekol and co-workers (14, 15, 16) using pure, crystalline vitamin B_{12} in place of the crude liver extracts and either homocystine or homocysteine as the only sulfur-containing amino acids.

In view of the synthesis of methionine (and, therefore, of methyl groups) which must have occurred, Stekol and Weiss (16) concluded that the precursors of the carbon of these methyl groups must have been present in the diet. They discussed the possible sources of this carbon, suggesting the amino acids — glycine and serine or their precursors — as the most likely contributors. They stated, "we are not implying that these amino acids are methyl group donors via metabolic degradation to intact methyl groups . . . we are suggesting that, in the course of metabolic interconversion of certain amino acids, carbon units are elaborated which serve as the carbon source for the synthesis *de novo* of the methyl groups of certain metabolites, including methionine and choline. Vitamin B_{12} and other dietary factors may play a decisive role in these interconversions, in the synthesis and utilization of the labile methyl group, and possibly in what is known as transmethylation."

Interpretation of Earlier Findings:

In the light of these newer findings it is possible to offer some explanation of the divergent findings reported in the first part of this Review.** As summarized by duVigneaud and co-workers (3):

---

*Also present in the medium were homocysteine (serving as a precursor for methionine) and dimethylaminoethanol (precursor of choline) and the vitamins, folic acid and B_{12}.

***cf. Borden's Review of Nutrition Research,* February, 1951 "I. Anti-Anemia Factors and the Biological Methylating Agents."

In the original paper in which it was shown that homocystine plus choline could serve in lieu of methionine for growth purposes in the rat, it was noticed that an occasional animal could grow without choline. It was inferred that these animals were provided with methyl groups synthesized by their intestinal bacteria. It is now obvious that the methyl groups were being synthesized chiefly by the tissues and not by bacteria in the intestine. The suggestion first put forth by the Lankenau workers that vitamin factors may be involved in the synthesis of labile methyl groups in the tissues is now seen to be the probable explanation. It is now realized that the diet used in the early work (at Cornell) was extremely low in folic acid and vitamin B_{12}. If vitamin B_{12} and folic acid bear a relationship to methyl metabolism in the tissues, the possibility exists that the occasional animal that did grow may have been able to do so because of *bacterial synthesis of vitamin factors*. This may have been the result of a difference in the bacterial flora or it may have been due to greater storage by such animals of B_{12} and folic acid from the pre-experimental diet. The more frequently encountered growth in the Lankenau Laboratories may be attributable, as suggested by the Lankenau workers, to the fact that their pre-experimental diet carried relatively large amounts of vitamin factors which may have been stored in the liver and later used in the experimental period. This is consistent with the now well-known storage of vitamin B_{12}. Of course, the pre-experimental diet, as these workers well realized, may also have affected the flora of the intestinal tract in such a way as to favor the synthesis of these vitamin factors.

Conclusion:

The newer studies on the biosynthesis and intermediate metabolism of labile methyl groups have re-emphasized the importance of these units in the body economy. The new light which has been shed on the relation of vitamin B_{12} and of folic acid to the biosynthesis of methyl groups may, eventually, be reflected to clarify the mechanisms whereby these vitamins function, not only in methyl synthesis, but in the complex picture of anemia as well.

With respect to the significance of labile methyl group biosynthesis in relation to the dietary requirement for these units, duVigneaud, Ressler and Rachele (3) have pointed out:

> "The experience at Cornell Laboratories has been that even when fairly high levels of B_{12} and folic acid are present a source of labile methyl groups must be present in the diet immediately after weaning for the majority of animals to survive. From the work of Griffith (17) it is known that the demand for choline is higher in the young rat shortly after weaning than later, to prevent hemorrhagic kidney. Undoubtedly some synthesis of the methyl group at this critical stage does take place but, in the majority of animals, apparently at a rate not fast enough to support normal development of the young animal. These results tend to place the biologically labile methyl groups in the same position that arginine holds with respect to the concept of the essential amino acids, in that it can be synthesized but not at a rate fast enough for the demands of the young growing rat. Thus,

in this sense, it can be regarded as an essential dietary component. A supply of preformed labile methyl groups in the diet may also become of considerable significance under special dietary or pathological conditions. Finally, it might be added that the behavior of the biologically labile methyl group that is synthesized within the body and used in the synthesis of a labile methyl compound is not distinguishable, so far as we know, from that of the biologically labile methyl group that is presented to the body in the diet from the standpoint of the metabolic process of transmethylation."

REFERENCES CITED

1. REYNIERS, J. A., and P. C. Trexler. The germ-free technique and its application to rearing animals free from contamination; in *Micrurgical and Germ-Free Methods.* Ed. by J. A. Reyniers, Chas. C. Thomas, Baltimore (1943).

2. REYNIERS, J. A., P. C. Trexler and R. F. Ervin. Rearing germ-free albino rats. *LOBUND Reports,* No. 1, Notre Dame, Indiana (1946).

3. DUVIGNEAUD, V., C. Ressler and J. R. Rachele. The biological synthesis of "labile methyl groups." *Science 112,* 267 (1950).

4. DUVIGNEAUD, V., M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds. The utilization of the methyl group of methionine in the biological synthesis of choline and creatine. *J. Biol. Chem. 140,* 625 (1941).

5. MELVILLE, D. B., J. R. Rachele and E. B. Keller. A synthesis of methionine containing radiocarbon in the methyl group. *J. Biol. Chem. 169,* 419 (1947).

6. MACKENZIE, C. G., J. P. Chandler, E. B. Keller, J. R. Rachele, N. Cross, D. B. Melville and V. duVigneaud. The demonstration of the oxidation *in vivo* of the methyl group of methionine. *J. Biol. Chem. 169,* 757 (1947).

7. MACKENZIE, C. G., J. P. Chandler, E. B. Keller, J. R. Rachele, N. Cross and V. duVigneaud. The oxidation and distribution of the methyl group administered as methionine. *J. Biol. Chem. 180,* 99 (1949).

8. MACKENZIE, C. G. Formation of formaldehyde and formate in the bio-oxidation of the methyl group. *J. Biol. Chem. 186,* 351 (1950).

9. SIEKEVITZ, P., and D. M. Greenberg. The biological formation of formate from methyl compounds in liver slices. *J. Biol. Chem. 186,* 275 (1950).

10. WELCH, A. D., and W. Sakami. Synthesis of labile methyl groups by animal tissues *in vivo* and *in vitro. Fed. Proc. 9,* 245 (1950).

11. DUVIGNEAUD, V., and W. G. Verly. Incorporation *in vivo* of C^{14} from labeled methanol into the methyl groups of choline. *J. Am. Chem. Soc. 72,* 1049 (1950).

12. DUVIGNEAUD, V., W. G. Verly and J. E. Wilson. Incorporation of the carbon of formaldehyde and formate into the methyl groups of choline. *J. Am. Chem. Soc. 72,* 2819 (1950).

13. SAKAMI, W. Acetone metabolism in the intact rat. *Fed. Proc. 9,* 222 (1950).

14. STEKOL, J. A., *et al.* Abstracts, Am. Chem. Soc. 116th meeting, 55C, Sept. 18 (1949).

15. STEKOL, J. A., M. A. Bennett, K. Weiss, P. Halpern and S. Weiss. Sulfur-containing amino acids in growth with a "labile-methyl"-free diet containing vitamin B_{12}. *Fed. Proc. 9,* 234 (1950).

16. STEKOL, J. A. and K. Weiss. Vitamin B_{12} and growth of rats on diets free of methionine and choline. *J. Biol. Chem. 186,* 343 (1950).

17. GRIFFITH, W. H. Choline metabolism. IV. The relation of the age, weight and sex of young rats to the occurrence of hemorrhagic degeneration on a low choline diet. *J. Nutr. 19,* 437 (1940).

NEWS DIGEST

VITAMIN DEFICIENCY ASSOCIATED WITH ANTIBIOTIC THERAPY: Preliminary clinical reports during the last year indicate that prolonged treatment of infections with a mixture of antibiotics (aureomycin and chloromycetin) may result in severe side effects characteristic of multiple vitamin deficiency. In one series of studies, in addition to the gastrointestinal symptoms of nausea and/or vomiting, heartburn, epigastric distress and diarrhea, it was noted that the stools often became odorless. This was attributed to a change in normal intestinal flora. Changes in condition of the mucous membranes, closely resembling riboflavin deficiency, were also noted. The lesions failed to respond to oral vitamin therapy, but did improve after parenteral administration of a potent B complex preparation. Minor bleeding tendencies in various areas were reported and thought to be associated with a possible deficiency of vitamin K. Skin lesions in the perineal region resulting from fungus infection (with Candida albicans) was another finding. This was likewise attributed to the destruction of normal intestinal flora which ordinarily inhibit the growth of such fungi.

In another case report, similar symptoms were noticed. Besides these, there were manifestations of peripheral neuritis, optic neuritis, severe gastrointestinal bleeding (associated with prothrombin deficiency), lesions of the tongue and of the skin of the nose, ear and armpits. Liver enlargement and slight impairment of liver function were also found. The investigators stated:

> "The conclusion that these disturbances resulted from the use of aureomycin and chloramphenicol* appears warranted from the sequence of events and is supported by the reported experiences of others. Since both antibiotics were administered concurrently, neither can be held solely responsible."

These studies emphasize the great importance of providing more than liberal supplies of B vitamins, and probably vitamin K, immediately following any protracted course of treatment with antibiotics, preferably via injection. — *Yale J. Biol. & Med. 23* 332 (1951).

PHILIPPINE NUTRITION PROGRAM: Almost exclusive use of white rice fortified with added thiamine, niacin and iron was introduced on October 1, 1948 among 63,000 people comprising the population of the east coast of Bataan in the Philippines.** The remainder of the province served as a control area receiving the customary white rice. Comparable clinical surveys of this population, plus that of two other towns in the control area, were conducted during the latter half of 1947 and the latter half of 1949. About 12,000 people were examined in each survey, about 88% of the total number being individuals who were inspected in both surveys. The incidence of beriberi symptoms among these people declined 89% in the second survey as compared with the first in the experimental area. In the control area (29,000 people) the incidence of beriberi rose slightly during the same period. Beriberi mortality has progressively declined in the experimental area from 263 per 100,000 in 1947-48 to 28 per 100,000 in 1949-50. No deaths from beriberi were reported from April 1 to June 30, 1950. The mortality from infantile beriberi declined 53% in the experimental area from 1947-48 to 1948-49.—*J. Nutr. 42* (1950).

---

*Chloromycetin

***cf. Borden's Review of Nutrition Research*, "Nutrition Highlights—1949", Jan. 1950